



# Kongeriget Danmark

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**Titel:** Retrovirale vectorer og deres anvendelse.

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**Viral vectors carrying SDI-1 or antisense SDI-1 genes under transcriptional control of target cell specific regulatory sequences or X-ray inducible promoters, and their use.**

The present invention relates to targeted expression of SDI-1 or antisense SDI-1 genes, and especially the use of the rodent WAP (Whey Acidic Protein) and the MMTV (Mouse Mammary Tumor Virus) regulatory sequences for targeted expression of linked SDI-1 or antisense SDI-1 genes in human mammary carcinoma cells.

**Background of the Invention**

Mammary carcinoma is the most frequent tumour in women (Miller and Bulbrook, 1986). Up to now the conventional therapy involves surgical removal of the primary tumour followed by a chemo- or radiation therapy. Depending on the tumour stage, the rate of relapse is quite high and has a fatal outcome in most cases. A major problem is the elimination of all metastases and micrometastases. Both this, as well as the serious side effects for the patient caused by conventional treatment, favour the development of a gene therapy approach (for a review on gene therapy see Anderson, 1992). One such approach could involve the use of a modified retrovirus or retroviral vector, to specifically deliver therapeutic genes to mammary carcinoma cells. The therapeutic gene would either inhibit the proliferation of tumour cells or kill the tumour cells after infection (suicide or toxin genes). The great advantage of a viral system would be that the virus particles can be spread in the blood stream similarly to metastasising tumour cells, which will make it possible to eliminate micrometastases long before they can be detected by conventional methods. Systemic delivery however poses the problem of the ability to target the therapeutic retroviral vector's expression only to the tumour cells. Therefore a control element is required to ensure that the transferred retroviral vector is only active in tumour cells.

Retroviral vector systems consist of two components:

- 1) the retroviral vector itself is a modified retrovirus (vector plasmid) in which the genes encoding for the viral proteins have been replaced by therapeutic genes and/or marker genes to be transferred to the target cell. Since

the replacement of the genes encoding for the viral proteins effectively cripples the virus it must be rescued by the second component in the system which provides the missing viral proteins to the modified retrovirus.

The second component is:

2) a cell line that produces large quantities of the viral proteins, however lacks the ability to produce replication competent virus. This cell line is known as the packaging cell line and consists of a cell line transfected with one or more plasmids carrying the genes enabling the modified retroviral vector to be packaged.

To generate the packaged vector, the vector plasmid is transfected into the packaging cell line. Under these conditions the modified retroviral genome including the inserted therapeutic and marker genes is transcribed from the vector plasmid and packaged into the modified retroviral particles (recombinant viral particles). This recombinant virus is then used to infect target cell's in which the vector genome and any carried marker or therapeutic genes becomes integrated into the target cell's DNA. A cell infected with such a recombinant viral particle cannot produce new vector virus since no viral proteins are present in these cells. However the DNA of the vector carrying the therapeutic and marker genes is integrated in the cell's DNA and can now be expressed in the infected cell.

A major consideration when considering the use of systemic retroviral delivery gene therapy, both from a safety stand point and from a purely practical stand point is, as mentioned above the targeting of retroviral vectors. It is clear that therapeutic genes carried by vectors should not be indiscriminately expressed in all tissues and cells, but rather only in the requisite target cell. This is especially important if the genes to be transferred are toxin genes aimed at ablating specific tumour cells. Ablation of other, nontarget cells would obviously be very undesirable.

A number of retroviral vector systems have been previously described that should allow targeting of the carried therapeutic genes (reviewed in Salmons and Gunzburg, 1993). Most of these approaches involve either limiting the infection event to predefined cell types or using heterologous promoters to direct expression of linked heterologous therapeutic or marker genes to specific cell

types. Heterologous promoters are used which should drive expression of linked genes only in the cell type in which this promoter is normally active.

In danish patent application no. 1017/94 the principle and construction of a new type of retroviral vector, the ProCon-vector, carrying various types of tissue specific regulatory elements are described:

The retroviral genome consists of an RNA molecule with the structure R-U5-gag-pol-env-U3-R. During the process of reverse transcription, the U5 region is duplicated and placed at the right hand end of the generated DNA molecule, whilst the U3 region is duplicated and placed at the left hand end of the generated DNA molecule. The resulting structure U3-R-U5 is called LTR (Long Terminal Repeat) and is thus identical and repeated at both ends of the DNA structure or provirus (Varmus, 1988). The U3 region at the left hand end of the provirus harbours the promoter and transcriptional regulatory sequences (see below). This promoter drives the synthesis of an RNA transcript initiating at the boundary between the left hand U3 and R regions and terminating at the boundary between the right hand R and U5 region. This RNA is packaged into retroviral particles and transported into the target cell to be infected. In the target cell the RNA genome is again reverse transcribed as described above.

In the ProCon-vector the right-hand U3 region is altered (Fig. 1), but the normal left-hand U3 structure is maintained (Fig. 1); the vector can be normally transcribed into RNA utilizing the normal retroviral promoter located within the left-hand U3 region (Fig. 1). However the generated RNA will only contain the altered right-hand U3 structure. In the infected target cell, after reverse transcription, this altered U3 structure will be placed at both ends of the retroviral structure (Fig. 1).

If the altered region carries a polylinker (see below) instead of the U3 region then any promoter, including those directing tissue specific expression such as the WAP promoter (see below) can be easily inserted. This promoter can then be utilized exclusively in the target cell for expression of linked genes carried by the retroviral vector. Additionally DNA segments homologous to one or more cellular sequences can be inserted into the polylinker for the purposes of gene targeting, by homologous recombination. Other means of directing gene expression to target tissue is to use X-ray inducible promoters.

The expression vectors used for the purpose of the invention need not be of the ProCon type, but can be any conventional vector carrying heterologous DNA sequence(s) under transcriptional control of the WAP or MMTV regulatory sequences. The vector used for the purpose of the invention can be a retroviral vector of conventional type i.e. with the WAP or MMTV promoters used as internal promoters, i.e. LTR-neo-WAP-therapeutic-gene-LTR, but is most preferably a retroviral vector of the ProCon type.

Vector constructs carrying various types of mammary gland specific regulatory elements have been tested in mice where expression of a marker gene driven by the regulatory elements in the hormonally stimulated mammary gland could be achieved (DK patent application no. 1017/94). One regulatory element demonstrated to give rise to expression in the pregnant and lactating mouse mammary gland is a small region of the rodent WAP promoter (Kolb et al., 1994). This gene is only expressed in the pregnant and lactating mammary glands of rodents and has no human homologue (Hennighausen, 1992). It is therefore not predictable that this regulatory element will function at all to direct expression in human cells and/or allow expression in human mammary carcinoma cells.

It was thus quite unexpected when the inventors of the present invention found that a 578bp element of the WAP promoter is able to direct expression of a linked marker gene ( $\beta$ -gal) in primary human mammary carcinoma cells.

The therapeutic gene to be delivered to the tumor cells is another important element in the construction of viral vectors for use in cancer therapy, and here the "Senescent Derived Inhibitor" SDI-1 and the corresponding SDI-1 gene and antisense SDI-1 genes are of particular interest:

Dividing cells undergo a cyclical programme that culminates in cell division. A normal event in this cell cycle programme is the replication of the cellular DNA. Just prior to this DNA synthesis, there is a pause to allow proof reading of the DNA, ensuring that any damage or mutations are repaired and not passed on to daughter cells. This checkpoint is regulated by programmed gene expression. A second, similar checkpoint occurs later on after DNA synthesis, just before the cell divides into two new cells, presumable for the same purpose. Senescent or aged cells are permanently arrested at one of these checkpoints. Recently, Olivia Pereira-Smith, Jim

Smith and colleagues have identified three cDNAs that cause growth arrest when transfected into young, actively dividing cells (Noda et al., 1994). One of these sequences, SDI-1 has also been independently cloned by other groups as a cyclin-dependent kinase inhibitor (CIP1; Harper et al., 1993), a gene that is induced by p53 (WAF1; El-Diery et al., 1993) and a gene involved in melanocyte differentiation (MDA6; Jiang and Fisher, 1993). Thus the same gene has a central role in cellular processes that have in common the loss of cell proliferation which implicates this gene as being involved in cell cycle control. SDI-1 has been shown to be overexpressed in senescent cells, quiescent cells or cultured primary cells undergoing crisis (Noda et al., 1994; Rubelji et al., 1994), suggesting a role in the maintenance of DNA synthesis inhibition (Johnson et al., 1994). Further, evidence has been presented suggesting that the SDI-1 mediated inhibition of DNA synthesis occurs via an inhibition of Cdk activity (Nakanishi et al., 1995). These findings, together with the demonstration that SDI-1 can inhibit cell growth of young dividing cells, demonstrates that this gene will be useful for gene therapy to inhibit the growth of rapidly proliferating cells in diseases such as restenosis, in which smooth muscle cells inappropriately divide, or various cancers.

To achieve this, a cDNA encoding SDI-1 would be placed under the control of a promoter in an expression cassette and delivered by any standard gene transfer technique, preferably in a retroviral vector. Rapidly dividing cells would then be infected with the vector. A retroviral vector based upon murine leukemia virus would offer the advantage that they are only able to successfully deliver genes to dividing cells, thereby avoiding that surrounding nondividing cells are infected. The infected cells would then express SDI-1 and become arrested in the cell cycle, preventing further cell divisions and possibly inducing senescence.

WO patent application No. 95/06415 describes the sequence of the SDI-1 gene and numerous therapeutical uses of the gene and the encoded protein, as well as antisense nucleotides capable of inhibiting the expression of the SDI-1 gene. The potential use of the SDI-1 gene and the encoded protein in cancer treatment is described in on pages 48-57 of the specification. Here it is suggested that the SDI-1 gene or protein is used in combination with conventional chemotherapy:

The premise of chemotherapy is that cancer cells grow more rapidly than normal cells, and hence are more sensitive to cytotoxic agents than normal cells. Many chemotherapeutic agents exert their effect during a specific phase or set of phases of the cell cycle. And because only a fraction of tumor cells are in a specific phase at any given time, such drugs must generally be provided in repeated administration. The use of the SDI gene or protein to synchronize or maximize the percentage of cells that are in a particular phase of the cell cycle at the time of administering the chemotherapeutic, provides a means to increase the effectivity of chemotherapy.

For the delivery of the SDI-1 gene or antisense gene to cells it is suggested to use viral or retroviral vectors and to use tissue specific promoters in order to confine the therapeutic effect to a desired site or tissue (pages 59 and 67).

WO-A1-95/06415 do not however disclose the use of the mammary gland specific WAP and MMTV regulatory sequences for the expression of the SDI-1 gene and antisense SDI-1 genes.

### **Summary of the Invention**

The invention then, inter alia, comprises the following, alone or in combination:

A replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters or X-ray inducible promoters;

a replication-defective retroviral vector as above, wherein the vector comprises a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence containing the target cell specific regulatory elements or promoters or an X-ray inducible promoter, followed by the U5 and R region,



characterized in that at least one of the coding sequences is a SDI-1 gene or an antisense SDI-1 gene;

a replication-defective retroviral vector as any above, wherein the target cell specific regulatory element is the WAP or MMTV regulatory sequences;

a replication-defective retroviral vector as above, wherein the regulatory sequence is the 578bp element of the WAP promoter-HGH gene hybrid or any other element/region of the WAP regulatory sequence conferring mammary specific expression;

a replication-defective retroviral vector as above, wherein the regulatory sequence is the U3 region of MMTV or subregions thereof conferring mammary specific expression;

a packaging cell line preferably of rodent, canine, feline or human origin or a packaging cell line histocompatible with human tissue harbouring:

- 1) a retroviral vector as any above
- 2) at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged;

a recombinant vector virus particle obtained by culturing the packaging cell line as above under suitable conditions optionally followed by isolation of the recombinant vector virus produced;

a pharmaceutical composition comprising the recombinant vector virus particle as above or a packaging cell line as above;

a method for the treatment of breast cancer comprising administering to a human in need thereof a recombinant vector virus particle as above or a packaging cell line as above;

a method for the treatment of restenosis comprising administering to a human in need thereof a recombinant vector virus particle as above or a packaging cell line as above;

a retroviral provirus integrated in the human genome carrying a DNA-construct comprising a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter; and

a human cell, containing a DNA construct carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter.

The WAPgal and the MMTVgal constructs are of particular interest for the purposes of the present invention because the regulatory elements conferring tissue specificity are both derived from the rodent system. This may become an important safety feature because the use of human regulatory sequences in a retroviral vector could cause problems because homologous recombinations between the vector carried sequences and the corresponding cellular may cause genome instability.

The retroviral vector is based preferably either on a BAG vector (Price *et al.*, 1987) or an LXS vector (Miller and Rosman, 1989), but may be based on any other retroviral vector.

The retroviral vector preferably comprises a coding sequence selected from one or more elements of the group consisting of marker genes, and therapeutic genes.

Said marker genes are preferably selected from the group consisting of marker genes which codes for proteins such as  $\beta$ -galactosidase, neomycin, alcohol dehydrogenase, puromycin, hypoxanthine phosphoribosyl transferase (HPRT), hygromycin and secreted alkaline phosphatase

Another embodiment of the invention envisages the alteration or partial deletion of at least one retroviral sequence required for the integration of the retrovirus.

The term "SDI-1 gene" means any nucleotide sequence coding for the SDI-1 protein, e.g. SDI-1 cDNA, or any derivative thereof capable of inducing cellular quiescence, including nucleotide sequences that codes for only a fraction of the SDI-1 protein, e.g. amino acid residues 1-70, 5-70, 10-70, 15-70, 20-70, 25-70, 30-70, 35-70, or 40-70.

The term "antisense SDI-1 gene" means any nucleotide capable of directing the synthesis of an RNA that can inhibit the expression of SDI-1 by formation of a triplex structure.

The packaging cell line is preferably selected from an element of the group consisting of  $\Psi$ -2,  $\Psi$ -Crypt,  $\Psi$ -AM, GP+E-86, PA317 and GP+envAM-12, or of any of these transfected with recombinant constructs allowing expression of surface proteins from other enveloped viruses.

A further embodiment of the invention provides therapeutical method for introducing the SDI-1 gene or antisense SDI-1 genes into human cells *in vitro* and *in vivo* comprising transfecting a packaging cell line of a retroviral vector system with a retroviral vector carrying one of these heterologous DNA sequences under transcriptional control of the WAP or MMTV regulatory sequences and infecting a target cell population with recombinant retroviruses produced by the packaging cell line.

According to the invention the term "polylinker" is used for a short stretch of artificially synthesized DNA which carries a number of unique restriction sites allowing the easy insertion of any promoter or DNA segment. The term "heterologous" is used for any combination of DNA sequences that is not normally found intimately associated in nature.

The following example will illustrate the invention further. The example is however in no way intended to limit the scope of the present invention as obvious modifications will be apparent, and still other modifications and substitutions will be apparent to anyone skilled in the art.

The recombinant DNA methods employed in practicing the present invention are standard procedures, well known to those skilled in the art, and described in detail, for example, in "Molecular Cloning" (Sambrook et al. 1989) and in "A Practical Guide to Molecular Cloning" (Perbal, 1984).

### Example 1

Mammary gland specific expression after infection with ProCon Vectors carrying mammary specific promoters.

In the murine leukemia virus (MLV) retroviral vector known as BAG (Price et al., 1987) the  $\beta$ -galactosidase gene is driven by the promiscuous (i.e. non-tissue specific) MLV promoter in the U3 region of the LTR (Fig. 1). According to the present invention a derivative of the BAG vector has been constructed in which the MLV promoter (U3) located within the 3'LTR (Fig. 1) has been deleted by PCR. At this position a polylinker was inserted containing the restriction sites *SacI* and *MluI* allowing the facile introduction of heterologous promoters. The BAG vector lacking the U3 is expressed from the MLV promoter (U3) within the 5'LTR when introduced into a packaging cell line. As a result of the usual rearrangements occurring in the retroviral genome during its life cycle, following infection of its target cell, the polylinker will be duplicated at both ends of the retroviral genome as described in danish patent application no. 1017/94. Thereby a retroviral vector can be constructed in which the expression of the  $\beta$ -galactosidase gene of BAG in the target cell will be controlled by any heterologous promoter inserted into the polylinker (Fig. 1).

According to the principle set forth above the following specific promoters have been inserted into the polylinker region of the modified BAG vector:

The Mouse Mammary Tumour Virus (MMTV) U3-Region (mtv-2) without the inverted repeats, which contains the MMTV promoter as well as a region that confers responsiveness to glucocorticoid hormones and a region containing an element that directs expression to the mammary gland.

The Whey Acidic Protein (WAP) promoter - Human Growth Hormone hybrid (Kolb et al., 1994) encompassing the positions -447 to +131 (with the transcription initiation site defined as +1), contains an element which controls the

expression of WAP so that it is only produced in the mammary glands of pregnant and lactating rodents.

The control of the  $\beta$ -galactosidase gene expression by promoters inserted into the polylinker has been validated by infection studies using the constructed MMTV and WAP retroviral vectors to infect various cells.

To produce retroviral vector particles, the MMTV and WAP ProCon vectors have been transfected into the packaging cell line GP+E86 (Markowitz et al., 1988) or PA 317 (Miller and Buttimore, 1986). After selection for neomycin resistance, which is encoded by the vector, stable populations and clones of recombinant ProCon virus producing cells were obtained. These clones and populations were producing recombinant virus into the cell culture medium. Alternatively 2 to 3 days after transfection (without selection) cell culture supernatant containing recombinant virus was harvested. Virus containing supernatant was used to infect explanted normal primary human mammary tissue obtained from reduction mammaplasties. Since it is known that these promoters are responsive to pregnancy hormones, the tissue was cultivated in the presence of such hormones. The expression of the marker gene was determined by a quantitative  $\beta$ -gal assay which is based on the detection of  $\beta$ -galactosidase activity by chemiluminescence. In all the experiments the original, non-tissue specific BAG-vector was used as a positive control. All of the analysed samples showed  $\beta$ -galactosidase expression (Fig. 2) in three independent experiments. It has thus been demonstrated for the first time that the WAP regulatory elements as well as the MMTV-U3 region can drive the expression of a gene within a MLV retroviral vector in primary human mammary gland cells.

To determine whether these regulatory sequences are active in human mammary tumours as well, primary explants of human mammary tumours were infected with WAPgal. A few days later the tumour organoids were analysed for  $\beta$ -gal expression as in the experiments described above. In this experiment the human mammary tumour cells infected with the WAPgal retroviral vector showed  $\beta$ -gal expression (Fig. 3a). In another experiment it was demonstrated that the MMTVgal (125.gal) and the non-tissue specific BAG construct also express the  $\beta$ -

gal gene in primary normal human mammary cells(i.e. non tumor derived cells) (Fig. 3b).

### Example 2

Expression of the SDI-1 cDNA has been tested using SDI-1 delivered by a retroviral vector and expressed under the transcriptional control of the inducible MMTV promoter. The retroviral vector used is of the ProCon type and carries the SDI-1 cDNA downstream of the 5'LTR and the MMTV U3 region inserted into the polylinker in the 3'LTR (Fig. 4). The vector also carries a neomycin (G418) resistance gene under the transcriptional control of the SV40 promoter (Fig. 5). The vector pLXS-SDI-1, was constructed using SDI-1 cDNA generated by polymerase chain reaction on the plasmid pSDI-1 (Noda et al., 1994) using two primers positioned at the 5' and 3' ends of the cDNA carrying heterologous 5' extensions. The sequence of the left hand PCR primer is 5' TATGGACGTC -TCCCTGCCGAAGTCAGTT 3' and the sequence of the right hand primer is 5' TATGGGATCC - GGCAGAAGATGTA GAGCG 3' . The 5' extensions harbor the sequence for an *Aat* II or a *Bam* HI restriction site on the left hand and right hand primers respectively. After digestion of the generated PCR product with *Aat* II and *Bam* HI , the SDI-1 cDNA was inserted into the *Aat* II and *Bam* HI sites of the Procon vector p125 carrying the MMTV promoter to produce the plasmid p125.SDI, whereafter the 4.9 kb *Afl* II - *Aat* II fragment of this plasmid was ligated to the *Afl* III-*Eco* RI fragment of pLXSN after blunt ending of both fragments. After introduction of the vector into the packaging cell line PA 317, the vector virus produced was used to infect cells, in this case the human bladder carcinoma derived EJ cell line (Paranda et al., 1982). G418 resistant cell clones have been isolated and analysed for acquisition and expression of SDI-1 and for their growth properties. Because of the use of a ProCon vector, after the infection event, the SDI-1 is placed under the transcriptional control of the glucocorticoid inducible MMTV promoter. Clones that have acquired the SDI-1 gene show a reduced growth rate when SDI-1 gene expression is induced from the MMTV promoter by treatment of the cells with the synthetic glucocorticoid hormone, dexamethasone.

The ability of dexamethasone to turn on SDI-1 expression and the resulting growth inhibition has been examined in various ways:

Figure 6 shows the result of S1 analysis of expression from LXS-125 SDI-1 infected EJ cells; only infected cells synthesise the expected transcript detected as a 79 nucleotide (nt) fragment after treatment with dexamethasone. On longer exposures transcripts can also be detected from the same cells grown in the absence of dexamethasone. Roughly equal amounts of RNA were analysed as can be seen in lanes 5-8 examining the expression of a cell encoded gene GAPDH.

Figure 7 shows the result of fluorescent activated cell sorting of cells to determine the proportion of cells in the various stages of the cell cycle. EJ cells infected with the LXS-125 SDI-1 were grown in the presence of dexamethasone (+DEX) or in the absence of dexamethasone (-DEX). The cells were stained for DNA content and analyzed on a cell sorter. The percentage of cells in the various stages of the cell cycle is given on the figure. The treatment with dexamethasone results in a greater proportion of cells in G<sub>0</sub>/G<sub>1</sub> phase and correspondingly less cells in the S phase.

Figure 8 shows the inhibition of cell growth measured by Giemsa staining of cells. EJ cell clones infected with LXS-125 SDI (EJ LXS-SDI) and noninfected cells were seeded in multiwell plates (5,000 cells per well) and allowed to grow for 5 days either in the presence (+D) or in the absence (-D) of dexamethasone. The infected EJ cells but not the non-infected EJ cells grow slower in the presence of dexamethasone suggesting that the expression of SDI (and not a nonspecific action of the dexamethasone) is responsible for the reduced growth rate of these cells.

An inducible promoter would not necessarily have to be used in SDI-1 carrying retroviral vectors for eventual gene therapy, though it may be useful in some instances.

### Example 3

A second therapeutic use of SDI-1 involves the expression of an antisense SDI-1 to reduce the expression of endogenous SDI-1. This will prevent cells pausing to check DNA integrity and repair of the DNA before new DNA synthesis begins in preparation for the next cell division. If cells expressing antisense SDI-1 are treated with DNA damaging agents such as mutagens, carcinogens or irradiation (e.g. gamma, U.V.), the efficiency of DNA damage repair will be severely reduced because the cell will not pause to permit proof reading and repair. These cells will accumulate so much DNA damage that they are no longer viable. Again, it may be useful to be able to control the expression of the gene with inducible promoters.



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## Claims:

1. A replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters or X-ray inducible promoters.
2. A replication-defective retroviral vector according to claim 1, wherein the vector comprises a 5' LTR region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' LTR region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence containing the target cell specific regulatory elements or promoters or an X-ray inducible promoter, followed by the U5 and R region, characterized in that at least one of the coding sequences is a SDI-1 gene or an antisense SDI-1 gene.
3. A replication-defective retroviral vector according to claim 1-2, wherein the target cell specific regulatory element is the WAP or MMTV regulatory sequences.
4. A replication-defective retroviral vector according to claim 3, wherein the regulatory sequence is the 578bp element of the WAP promoter-HGH gene hybrid or any other element/region of the WAP regulatory sequence conferring mammary specific expression.
5. A replication-defective retroviral vector according to claim 3, wherein the regulatory sequence is the U3 region of MMTV or subregions thereof conferring mammary specific expression.
6. A packaging cell line preferably of rodent, canine, feline or human origin or a packaging cell line histocompatible with human tissue harbouring:
  - 1) a retroviral vector according to claims 1-5
  - 2) at least one retroviral or recombinant retroviral construct coding for

proteins required for said retroviral vector to be packaged.

7. A recombinant vector virus particle obtained by culturing the packaging cell line according to claim 6 under suitable conditions optionally followed by isolation of the recombinant vector virus produced.
8. A pharmaceutical composition comprising the recombinant vector virus particle according to claim 7 or a packaging cell line according to claim 6.
9. A method for the treatment of breast cancer comprising administering to a human in need thereof a recombinant vector virus particle according to 7 or a packaging cell line according to claim 6.
10. A method for the treatment of restenosis comprising administering to a human in need thereof a recombinant vector virus particle according to claim 7 or a packaging cell line according to claim 6.
11. A retroviral provirus integrated in the human genome carrying a DNA-construct comprising a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter .
12. A human cell, containing a DNA construct carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of the WAP or MMTV regulatory sequences or an X-ray inducible promoter.

**Abstract**

The present invention relates to a replication-defective retroviral vector carrying a SDI-1 gene or an antisense SDI-1 gene under transcriptional control of target cell specific regulatory elements or promoters.

# Construction of a U3 minus BAG-vector (MLV)

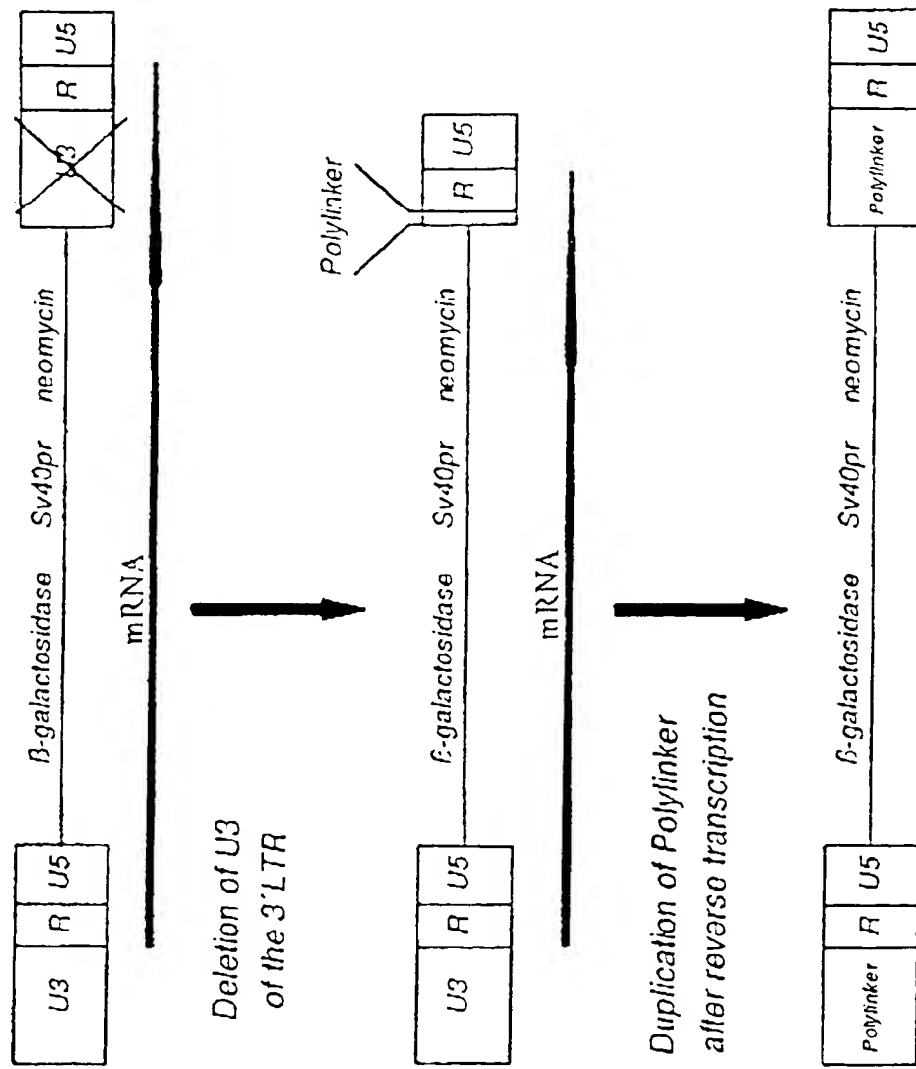


FIG 1

$\beta$ -Gal Expression of vector constructs after infection of primary hum. mgl cells

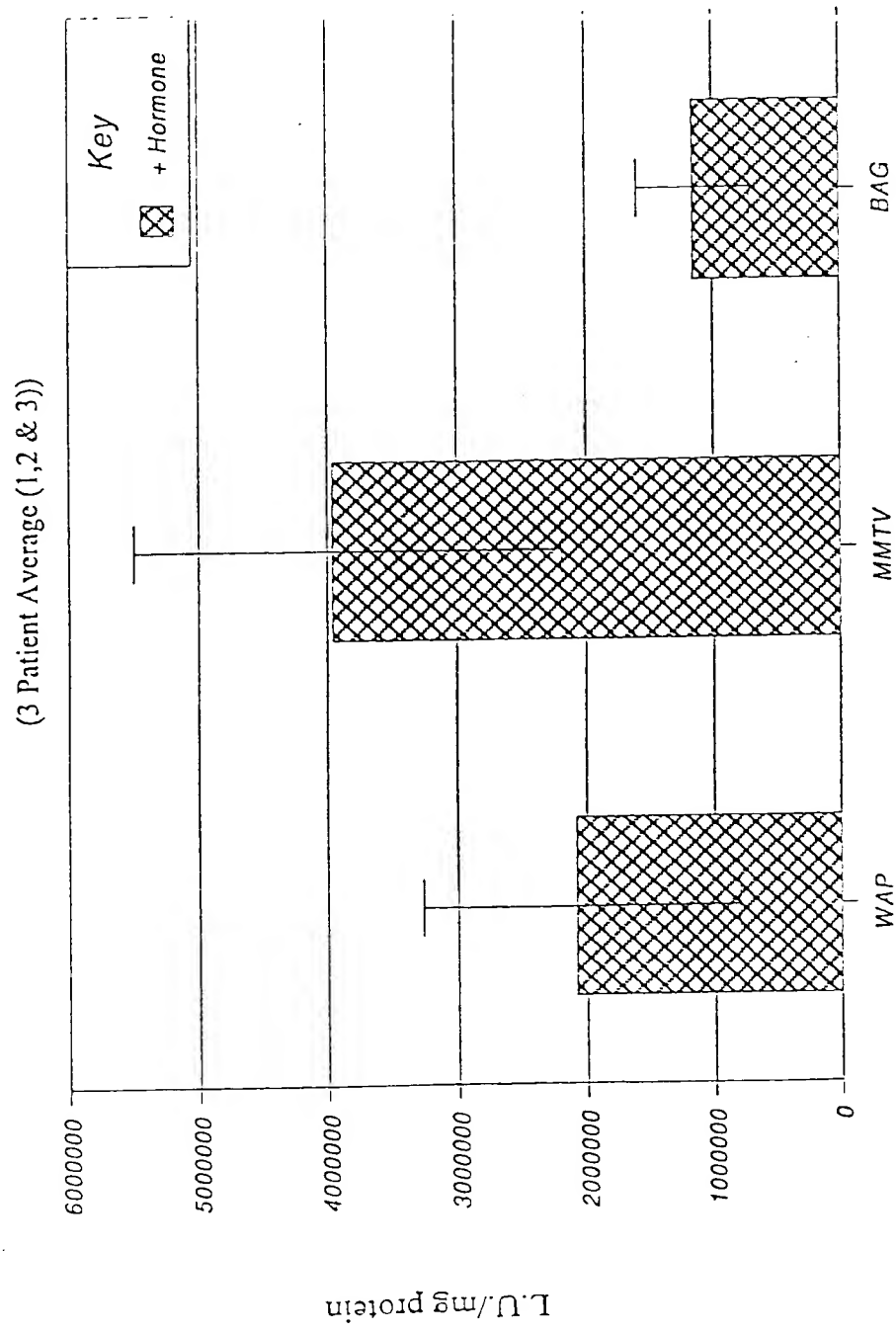


FIG 2

## Infection of 1° Human Mammary Carcinoma Cells

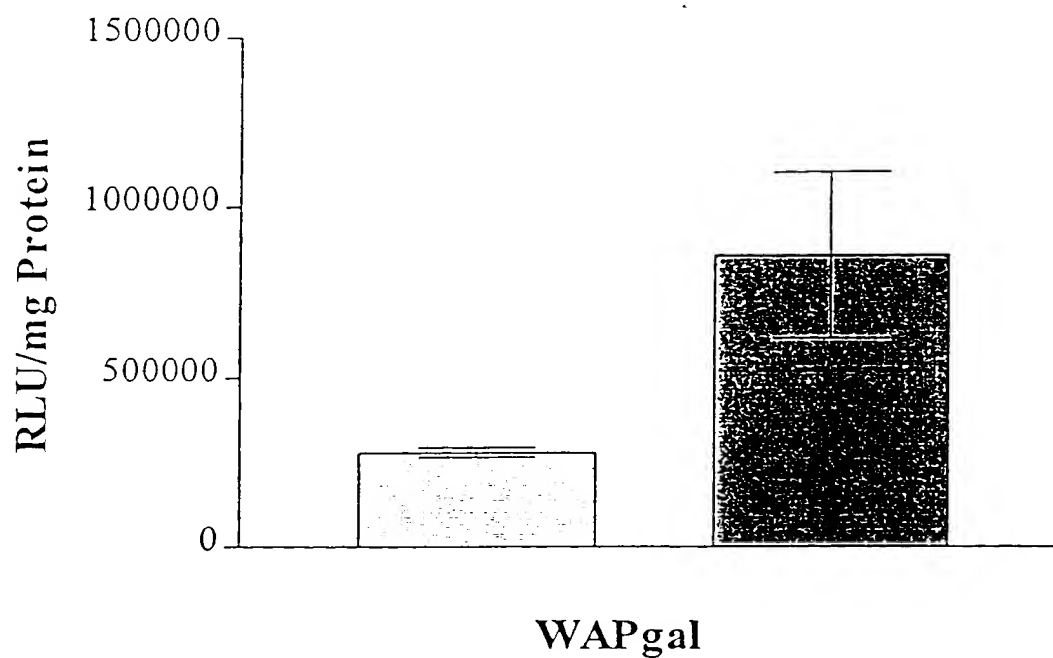


FIG 3.a

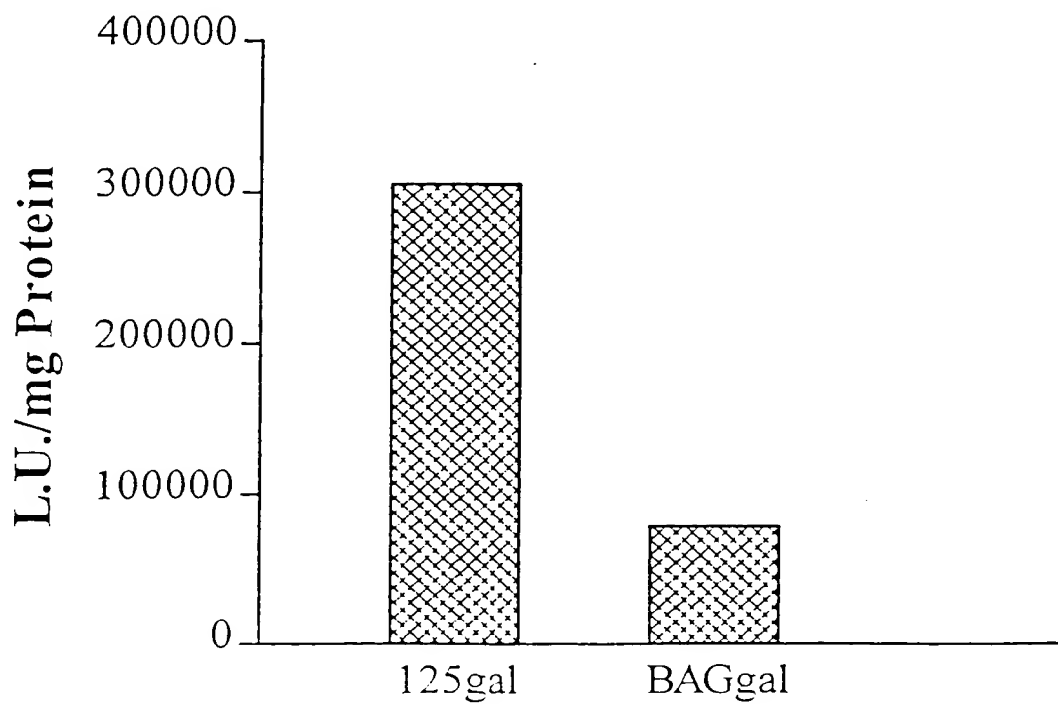


FIG 3.b





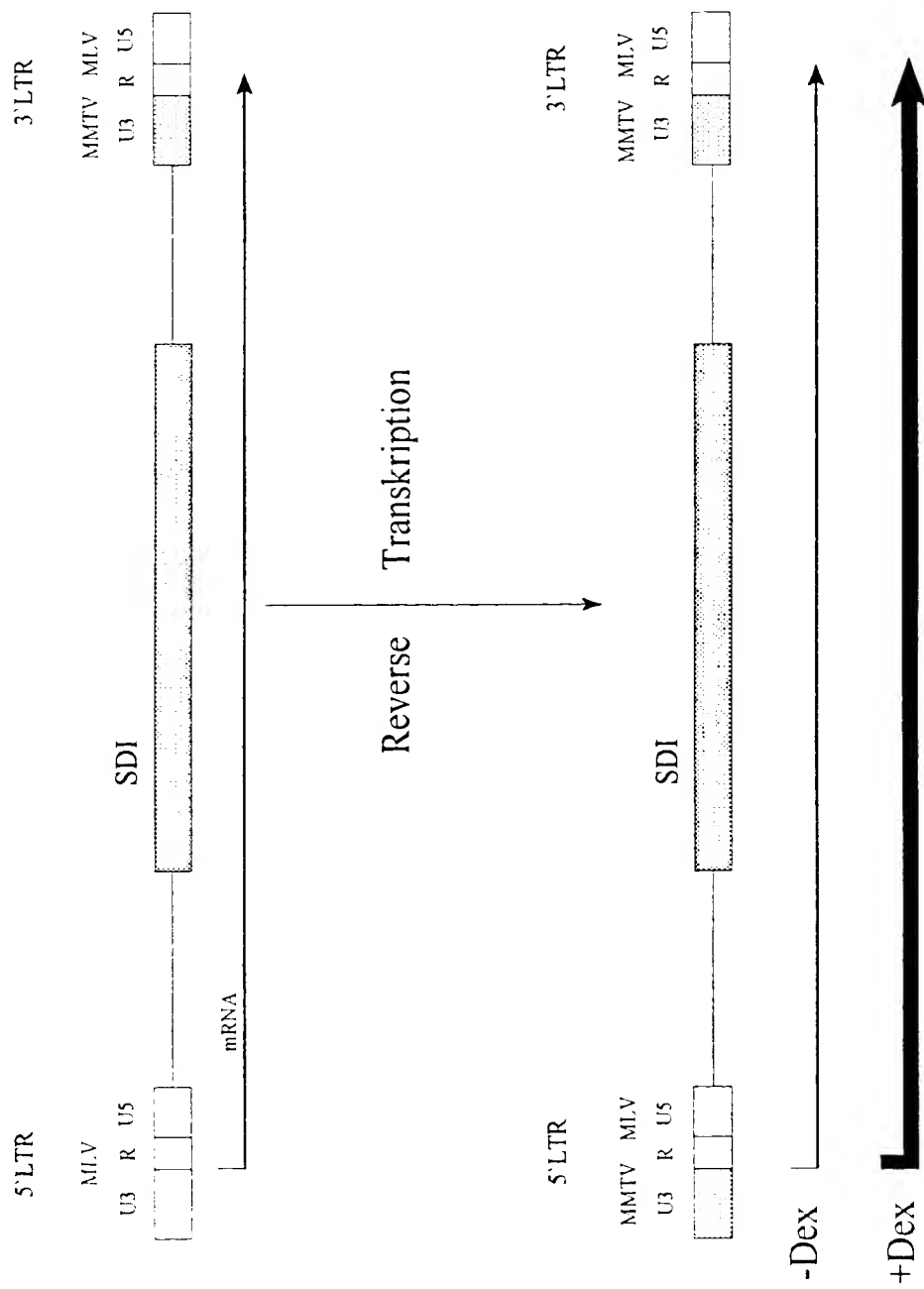
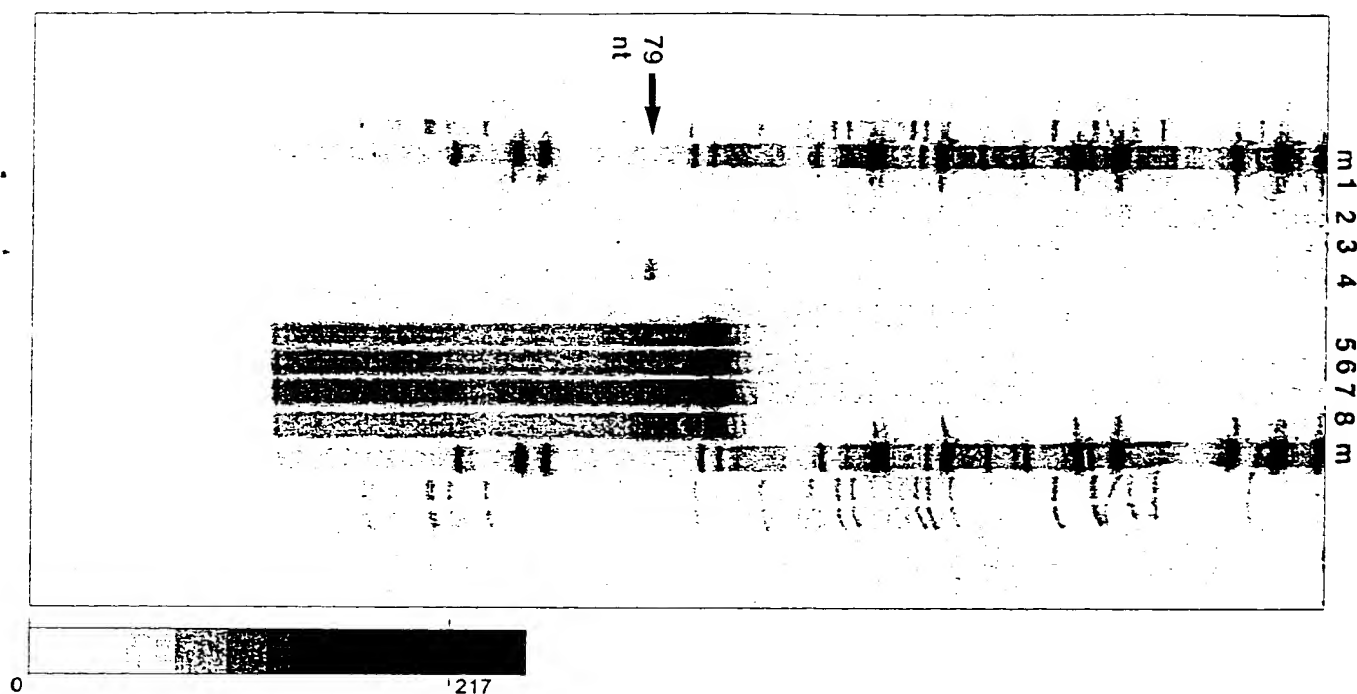


FIG 5



**FIG 6** - Analysis of Expression from LXS-125 SDI-Infected Cells

Lane M - standard marker fragments

Lanes 1 & 5 - RNA from EJ cells

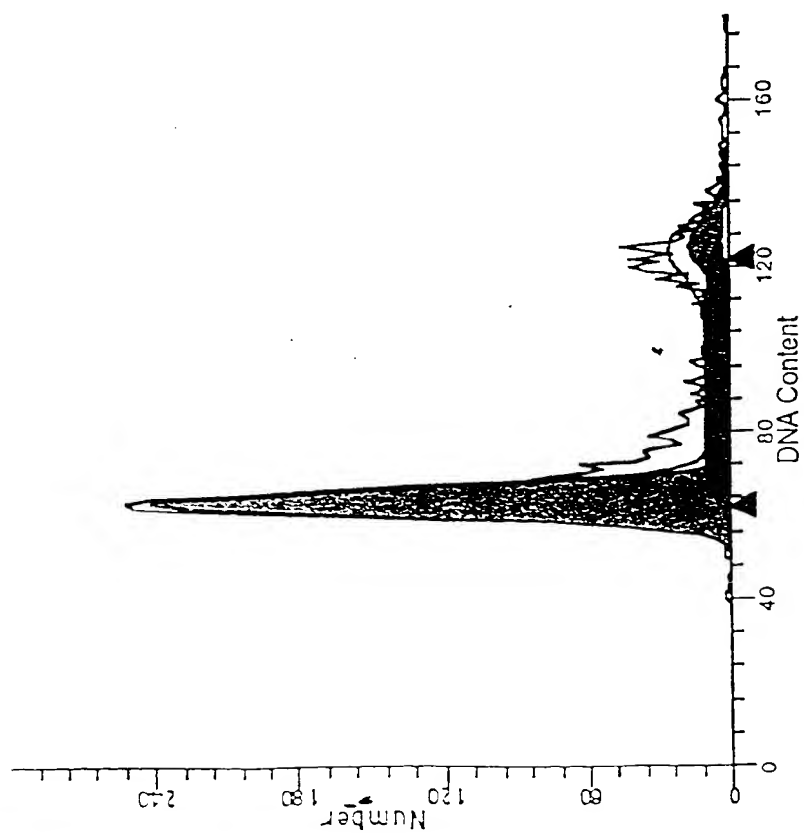
Lanes 2 & 6 - RNA from EJ cells treated with  $10^{-6}$ M dexamethasone

Lanes 3 & 7 - RNA from EJ cell clone infected with LXS-125 SDI

Lanes 4 & 8 - RNA from EJ cell clone infected with LXS-125 SDI  
treated with  $10^{-6}$ M dexamethasone

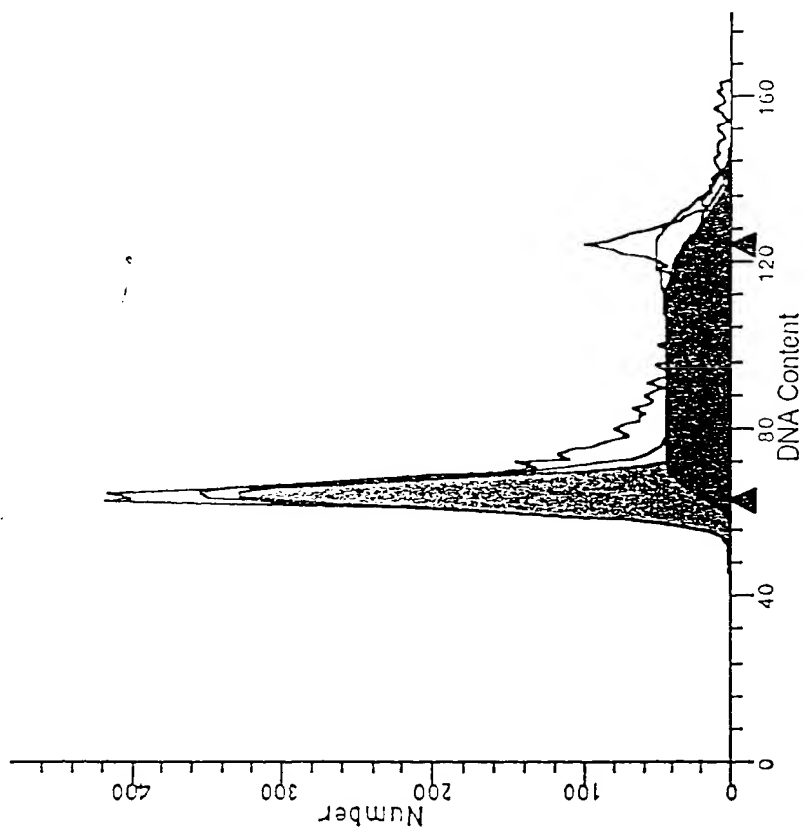
Lanes 1 - 4 using a probe specific for transcription from  
LXS-125 SDI

Lanes 5 - 8 using a probe specific for transcription from GAPDH



+DEX

|                               |      |
|-------------------------------|------|
| %                             |      |
| G <sub>0</sub> G <sub>1</sub> | 66.8 |
| G <sub>2</sub> M              | 9.6  |
| S                             | 23.6 |

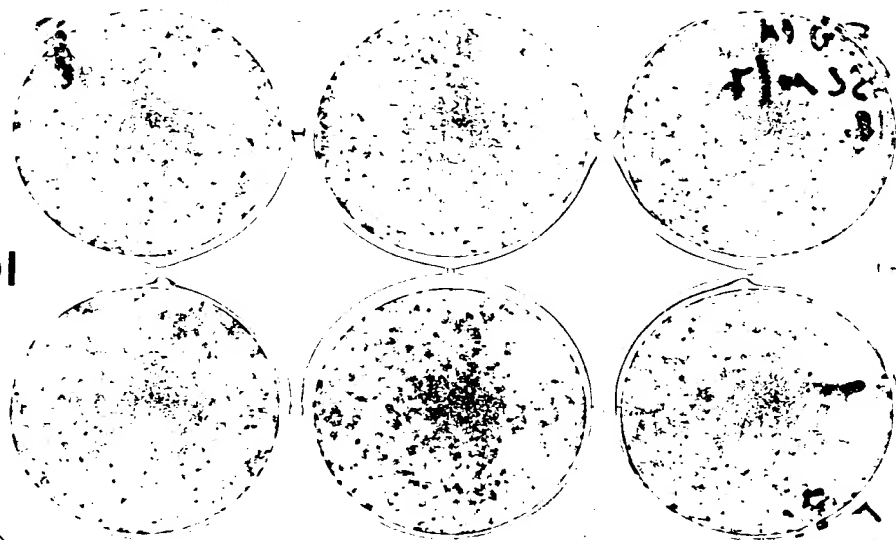


-DEX

|                               |      |
|-------------------------------|------|
| %                             |      |
| G <sub>0</sub> G <sub>1</sub> | 48.2 |
| G <sub>2</sub> M              | 6.4  |
| S                             | 45.4 |

FIG 7

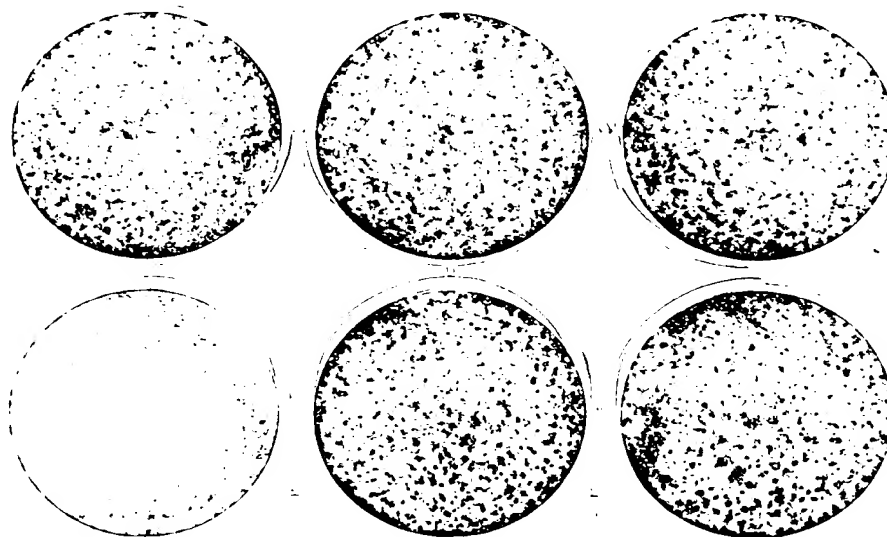
EJ  
LXS-SDI



+D

-D

EJ



+D

-D

FIG 8